

# Apoptosis

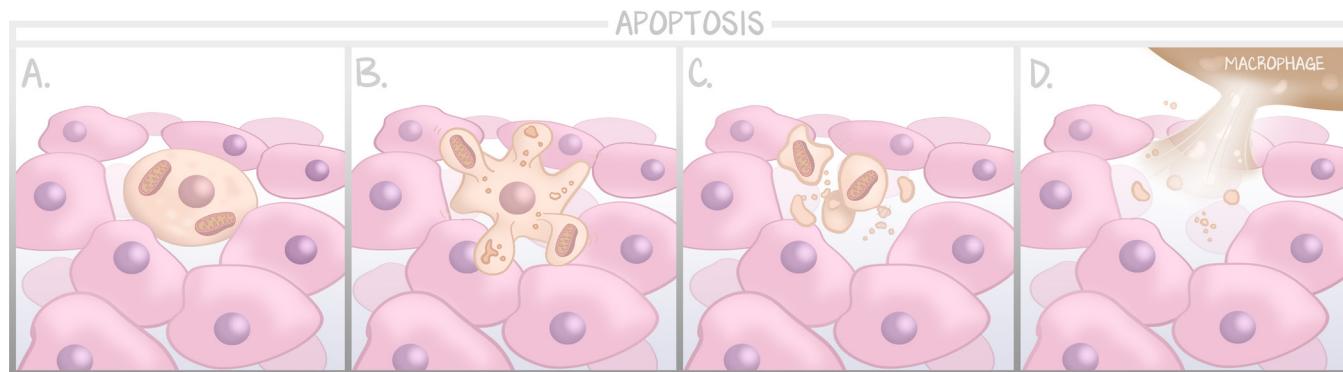
## Introduction

Apoptosis is **not cell suicide**. Everybody says “cell suicide,” but that can mislead the novice. Suicide can carry with it connotations of a gun blowing something apart, a sword spilling guts, or poison violently killing a person. That violent death is necrosis. Instead, think of apoptosis as the **programmed, systematic deconstruction of a cell**. In the same way a tech crew strikes a set after the play ends—storing all the cables together, hanging the lights in order of size and color, screws in their labeled boxes—so too does the cell disassemble and store its contents in apoptosis. When the next show comes through, everything is organized and in place for the next crew. The cell’s contents are recycled for use in other cells. No additional cleanup or reorganization is required. In this analogy, necrosis would be the equivalent of the stage crew’s lighting the building on fire when the show ended its run.

Apoptosis is the systematic disassembly of the cell and its parts, **contained by plasma membrane**, packaged for transport and recycling. It’s the reclamation of resources. It’s the magician who disappears into his own hat, vanishing on purpose, all part of the show. In apoptosis the **cytoplasm shrinks** because there is no loss of integrity of the cell membrane. **DNA material is packaged** and there is **no release of destructive enzymes**—the nearby cells do not feel the apoptotic cell’s disassembly. And because of that, there is **no immune response**, change in pH, or protein denaturation.

Apoptosis is kind of like a computer program in *The Matrix*. When the program isn’t needed anymore, it dissolves away as discrete 1’s and 0’s fluttering into the wind . . . the remainder still standing, doing what it was doing, until it’s entirely dissolved. Not a violent explosion, no lysis, no swelling. Just an effervescent disappearance without any impact on the nearby programs.

Well . . . what happens to these disintegrating particles of cells? Technically, a macrophage comes by and sweeps it up. There is no cytokine release. No inflammatory cells are summoned. You won’t see apoptosis on an histology slide because it is so subtle. Learn: apoptosis = no inflammatory cells.



**Figure 3.1: Apoptosis**

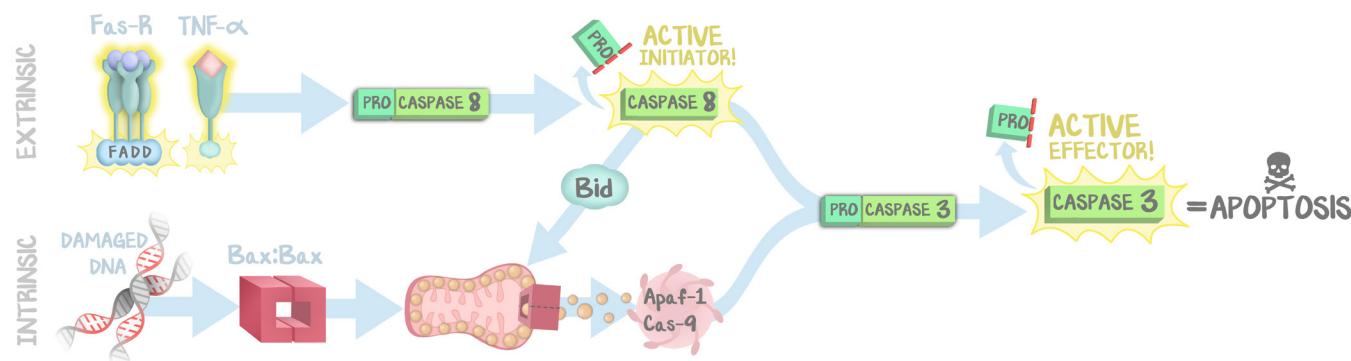
(a) A single cell is selected for apoptosis. (b) In a systematic fashion, organelles, cytoplasm, and eventually nuclear material bleb away from the disappearing cell bound in vesicles—plasma-membrane-bound structures. (c) The least needed go first, and eventually the rest systematically. (d) No cell death occurs, no inflammatory cells are involved, no cytoplasm leaks into the extracellular matrix. A single macrophage sweeps up the mess.

NECROSIS	APOPTOSIS
Large, contiguous patches of cells all dying together	A single cell (adult life) or small cluster (embryogenesis)
Swelling of cell membrane	Shrinkage
Disrupted membrane (lysis)	Intact membrane (no lysis)
Cytoplasm release causing lots of inflammation	Cytoplasm contained, so no inflammation
Pyknosis (gets smaller), karyolysis (fades), and karyorrhexis (fragments)	Pyknosis, karyolysis, but no karyorrhexis
Everything fragments	No fragmentation
Histology shows intact cytoarchitecture without proteins, ribosomes, or dark-staining material	Cell slowly takes itself apart, piece by piece

**Table 3.1: Necrosis vs. Apoptosis**

A comparison of the two events. Visualize necrosis as catching a grenade exploding everywhere. Very messy. Visualize apoptosis as the systemic, programmed dissolution of a cell in discrete packets.

We introduce a specialized kind of protein in this lesson that is responsible for apoptosis: caspases. Their name is derived from: cysteine-containing, aspartate-specific protease. C-asp-ase. For you “caspase” means “causes apoptosis” and you can ignore that cysteine, aspartate business. Caspases can exist in an inactive form, called **procaspase**. When the inhibitor portion of the procaspase is cleaved away, the remaining portion activates, and is called a **caspase**. Some are **initiator caspases**, which activate other intracellular mechanisms that eventually lead to apoptosis. Others are **effector caspases** (also called execution caspases); these directly induce apoptosis. Regardless of which mechanism is utilized, apoptosis will ultimately be mediated by caspases. Caspases also activate DNAases, RNAases, and other proteins, but as far as our learning of apoptosis is concerned, caspases are the end of the line. This mental divide facilitates keeping necrosis very separate from apoptosis, a necessary maneuver on our part since so many faculty members teach them at the same time.

**Figure 3.2: Caspases and Their Activation**

Apoptosis begins with caspase-3; when caspase-3 is cleaved from procaspase-3, apoptosis will occur. Caspase-3 can be activated by the intrinsic pathways, which lead to the release of cytochrome c from the mitochondria, which activates Apaf-1/caspase-9, which activates caspase-3. Caspase-3 can also be activated by the extrinsic pathways via caspase-8 (and an ancillary release of cytochrome c through Bid).

Caspase pathways are categorized as extrinsic, intrinsic, and common. This is not like the clotting cascade, where this nomenclature must be strictly memorized, but we explain the mechanisms as such here for completeness. **Extrinsic** pathways use **caspase-8** (death receptors, granzyme). **Intrinsic** pathways use **cytochrome c** and **caspase-9**. The **common** pathway is where caspase-8 and caspase-9 converge—the effector caspases, 3, 6 and 7. Eventually, either caspase-8 will activate effector caspase-3 or one of multiple mechanisms will activate the **mitochondrial cytochrome c** mechanism, which activates caspase-9, which activates caspase-3. Extrinsic-8-3, intrinsic-cyt c-9-3.

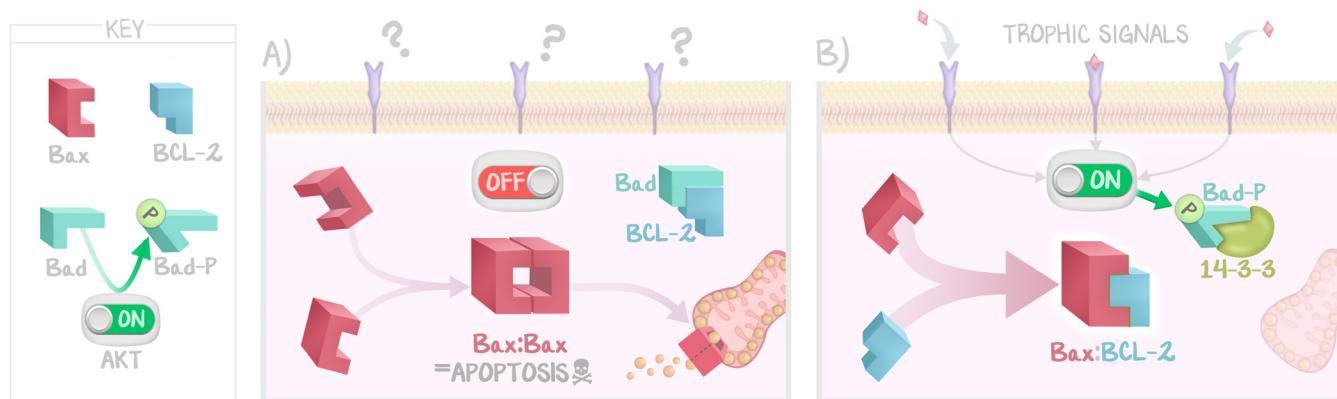
### Intrinsic Apoptosis: Bax, Bcl-2, Bad, and AKT

Cells require the host organism to want them around. If a cell isn't fed and given water, it will become depressed and lonely, activating apoptosis. When there are actively **expressed trophic factors** (the "stay alive! We love you!" signal), intracellular mechanisms **suppress apoptosis**. The absence of trophic factors causes the absence of apoptosis suppression, releasing apoptosis. One such mechanism involves Bax, Bad, Bcl-2, and AKT. This mechanism is important to understand, as similar players will be discussed in the subsequent *Intrinsic Apoptosis* sections.

The default of this system is for **Bax:Bax homodimers** to form a **mitochondrial pore** that allows the release of **cytochrome c** into the cytoplasm. Cytochrome *c* is usually a "good guy" in our cells, responsible for oxidative phosphorylation in the electron transport chain. But our cells, having become symbiotic with mitochondria, have also found a way to use the internal mitochondrial proteins to regulate our cells. When cytochrome *c* is released into the cytoplasm it activates caspases (Apaf-1 and caspase-9, the apoptosome), which eventually activates the **effector caspase, caspase-3**. The default of Bax genes is to make Bax protein, which combines with another Bax protein to make a pore that releases cyt *c*, which induces apoptosis. That default results in the cell's activation of cytochrome *c* and the induction of apoptosis.

**Bcl-2** is an **anti-apoptosis** gene. It produces the protein Bcl-2. Bcl-2 has a higher affinity for Bax than another Bax does. So when Bcl-2 is expressed, **Bax:Bcl-2 heterodimers** form, meaning the Bax:Bax pore does not form, and apoptosis is inhibited.

**Bad** is a **pro-apoptosis gene**. Bad comes in two forms, phosphorylated and dephosphorylated. **Bad-P** gets sequestered by protein 14-3-3. **Dephosphorylated Bad** escapes sequestration. Bad has a higher affinity for Bcl-2 than Bcl-2 has for Bax. So when Bad is free in the cytoplasm, Bad:Bcl-2 heterodimers form, preventing Bax:Bcl-2 heterodimers, thereby permitting Bax:Bax pores, allowing apoptosis.



**Figure 3.3: Failure of Trophic Signals**

(a) When trophic factors are absent, AKT is off, Bad is dephosphorylated and freed from 14-3-3, binding up Bcl-2, allowing Bax:Bax homodimers to form, leading to cytochrome *c* release and apoptosis. (b) When trophic factors are present, AKT is on, Bad is phosphorylated and sequestered by 14-3-3, and Bcl-2 prevents Bax:Bax homodimers from forming, preventing apoptosis.

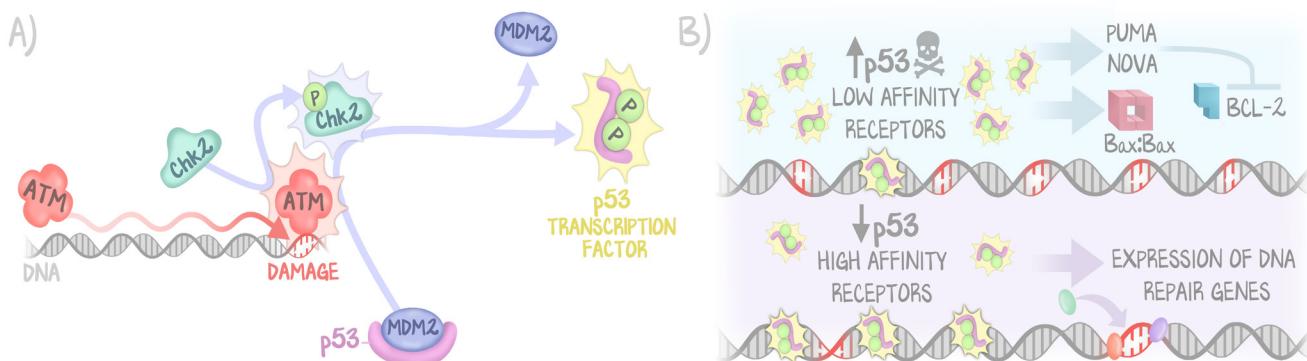
One means of regulating this system is by AKT, a protein that **phosphorylates Bad**. If AKT is on, Bad is phosphorylated, Bad doesn't bind Bcl-2, and Bcl-2 does bind Bax, so Bax:Bax cannot form. If AKT is off, Bad is dephosphorylated, Bad does bind Bcl-2, and Bcl-2 can't bind Bax, so Bax:Bax forms. **AKT is expressed as long as trophic factors are maintained.**

### Intrinsic Apoptosis: DNA Damage and p53

The cell cycle has checkpoints. It ensures that the DNA that will be propagated into future cell lines by a cell division is intact, complete, and without error. One of these checkpoint mechanisms is ATM-Chk2-MDM2-p53. **p53** is a **tumor suppressor gene** and is referred to as the guardian of the genome. It gets expressed when there is damaged DNA. In **mild** concentrations, p53 arrests the cell cycle while inducing expression of DNA repair genes. p53 both produces the repair gene product and gives those products time to work. In **moderate** concentrations, it shunts the cell into senescence, never to divide again. In **high** concentrations, p53 binds to a specific, low-affinity region of DNA. Its binding to this region of DNA actively **promotes apoptosis** by inducing expression of pro-apoptotic genes (like Bax), and of genes that inhibit anti-apoptosis genes or gene products (like PUMA, which inhibits Bcl-2).

This mechanism of apoptosis begins with the protein ATM. ATM surveys DNA for damage. If it encounters damaged DNA, it binds to and nicks the pentose-phosphate backbone of DNA. This nick will allow DNA repair enzymes to access damaged nucleotides. But more importantly to our discussion, the binding of ATM to damaged DNA self-activates the kinase function of ATM. Kinases add phosphates. ATM **phosphorylates Chk2** (activating it) AND **phosphorylates p53**. Chk2-P also phosphorylates p53.

**MDM2** sequesters p53. MDM2 can only sequester p53 when p53 is **not** phosphorylated. The phosphorylation of p53 by ATM and Chk2 causes the release of p53 from MDM2. The **more DNA damage there is, the more ATM activity there will be and the more p53 will be phosphorylated (disinhibited)**. The **longer it takes to repair the damage, the longer p53 will be active and the higher the concentration will get**.



**Figure 3.4: DNA damage and p53**

(a) Damaged DNA is detected by ATM. ATM binds to damaged DNA, activating its kinase activity, phosphorylating Chk2 and p53. Phosphorylated Chk2 also phosphorylates p53. Phosphorylated p53 is released from MDM2. (b) In high concentrations, p53 induces expression of Bax and induces expression of Nova and PUMA, which inhibit Bcl-2, thereby overall increasing Bax:Bax homodimer formation, cytochrome c release from the mitochondria, and ultimately apoptosis. Details are available in Lesson #6.

p53 acts as a **transcription factor** controlling the expression of genes that control apoptosis and cell arrest. It has multiple receptors. Low concentrations of p53 activate high-affinity receptors, which induces **cell-cycle arrest** and activates **gene repair**. High-affinity means “needs little p53 to activate.” Once fixed, p53 expression is reduced (because ATM no longer binds to damaged DNA) and the cell cycle continues. In the case of a lot of DNA damage, p53 expression is high because there is a lot of ATM bound to a lot of damaged DNA. This high concentration of p53 activates low-affinity receptors (these receptors need there to be a lot of p53 to bind), which induce apoptosis. Apoptosis is induced by the expression of Bax (so more Bax:Bax homodimers form) and inhibition of Bcl-2 (so fewer Bax:Bcl-2 heterodimers form).

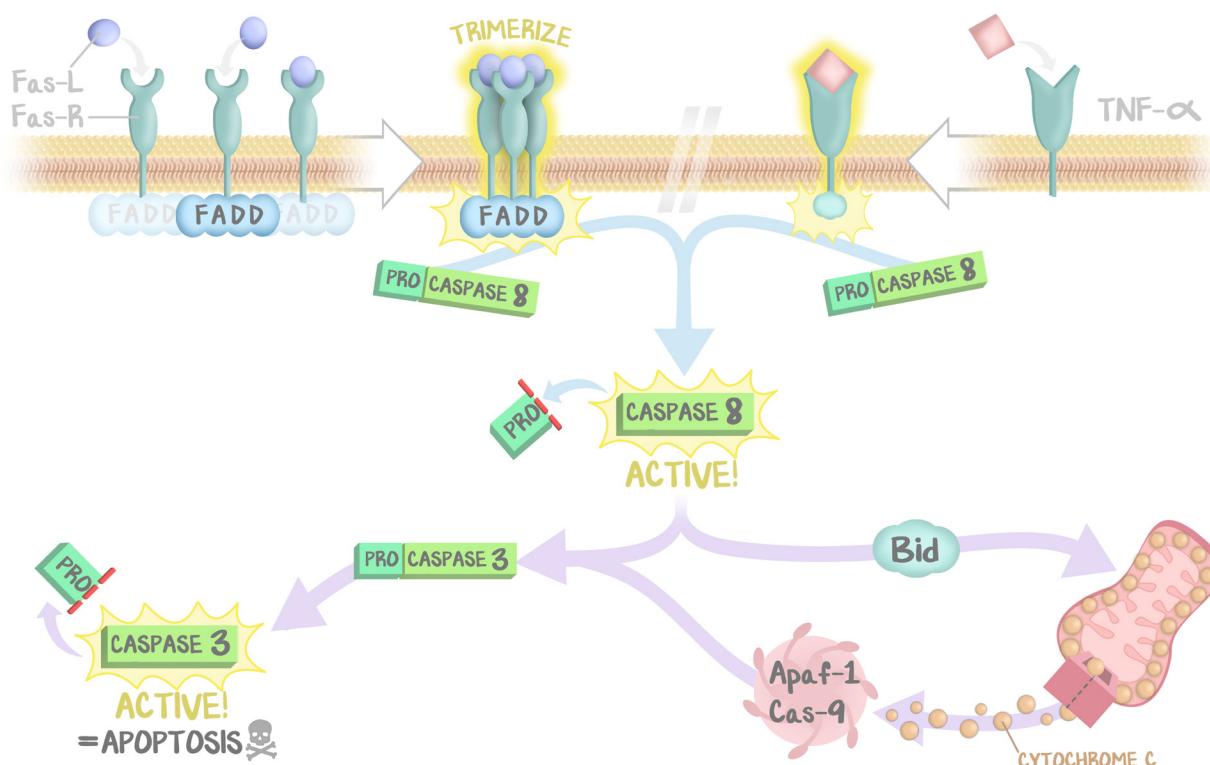
### Extrinsic Apoptosis: Fas-FasL, TNF- $\alpha$ , Perforins

An external signal from a cytotoxic (CD8+) T cell can induce a cell to undergo apoptosis. Any cell not behaving correctly could be infected with a virus or exhibiting the early signs of malignant transformation. It would be useful to eliminate that cell before its erratic behavior affects nearby cells or, worse, bad DNA is propagated via cell division. That elimination is apoptosis. Another reason to tell a cell to undergo apoptosis is because that cell is no longer needed (as in embryogenesis). Controlling the disassembly of that cell prevents the rupture and spilling of cytoplasmic contents.

There are three mechanisms whereby a host immune system can induce apoptosis in a cell: Fas-FasL, TNF- $\alpha$ , and perforin-granzymes. These are referred to as **death receptor activation** mechanisms. All result in the same final process: caspase-3-induced apoptosis. All get there using common pathways: caspase-8-mediated activation of caspase-3 and caspase-8-mediated activation of the intrinsic mitochondrial cytochrome *c* mechanism. In this process, caspase-8 is an **initiator caspase** (it doesn’t actually do the apoptosis but leads to caspase-3 activation) and caspase-3 is an **effector caspase** (it actually causes apoptosis).

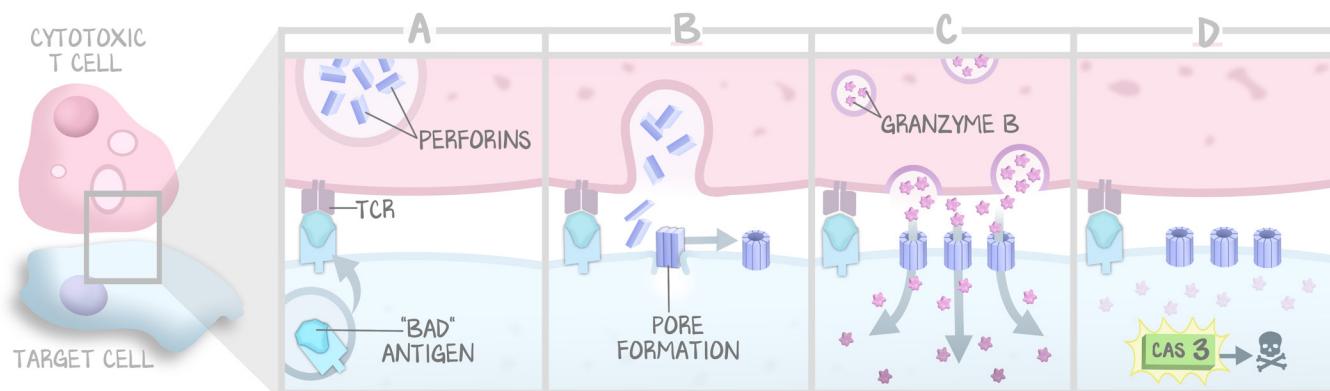
**Fas ligand (FasL)** binds the **Fas** receptor. Fas **trimerizes**. The trimer is the form of the active receptor. Trimerization causes activation of intracellular peripheral protein FADD. **Activated FADD** cleaves inactive **procaspase-8** to active **caspase-8**. Once active, caspase-8 has two mechanisms. The first is to cleave and activate **caspase-3**, the direct stimulator of apoptosis. The second is to **activate Bid**, which induces mitochondrial mechanisms (Bax:Bax homodimers, cytochrome *c*) which ultimately contributes to the activation of caspase-3.

**TNF- $\alpha$**  receptor activation works in a very similar way; consider it identical to Fas-FasL as it relates to apoptosis, except there is no trimerization—the **TNF- $\alpha$**  receptor is already a trimer.

**Figure 3.5: Extrinsic Apoptosis—Death Receptors**

Fas ligand (FasL) is released from cytotoxic cells, activating the Fas receptor. The Fas-FasL pathway results in trimerization, FADD association, and activation of caspase-8. TNF- $\alpha$  receptor activation has the same effect—caspase-8 activation—with trimerization. Activation of caspase-8 leads to activation of both caspase-3 (effector) and indirect activation of caspase-3 through Bid, which induces cytochrome c release from the mitochondria.

Cytotoxic T cells also have preformed **perforins** in vesicles which they can insert into the membrane of target cells. Perforins themselves are deadly, compromising the cell membrane and allowing ion/water exchange. This is a mechanism of lysing foreign cells. But because apoptosis is meant to be controlled cell deconstruction, lysis is not the purpose. Perforins are large enough channels to permit also the entrance of **granzymes**. Granzymes released by the cytotoxic T cell pass through the perforin into the cytoplasm of the target cell. **Granzyme B** also facilitates the activation of caspase-3 and Bid.

**Figure 3.6: Extrinsic Apoptosis—Granzymes and Perforins**

(a) Perforins are contained in vesicles in cytotoxic T cells. (b) Fusion of those vesicles with the cytotoxic T cell plasma membrane releases perforins which fuse in the target cell plasma membrane forming a pore. (c) Granzymes pass through the pore into the target cell, and (d) activate Bid and Caspase-3.